Soil nitrogen dynamics during stand development after clear-cutting of Japanese cedar (Cryptomeria japonica) plantations

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Author's Response to Reviewers' Comments

Reply to Editor's comment

In figure 2, regression line crosses y-axis. The regression line should be shorter or range of x-axis should be changed.

L35, L404

"…, which would cause biomass accumulation rate of Japanese cedar to decline in the older stand." This part should be deleted. The findings in this study dose not tell some mechanisms about decline of forest productivity in older stand.

Thank you for your comment. We have corrected all of them as indicated.

Article type: Original Article [Special feature: Soil nitrogen dynamics]

Title: Soil nitrogen dynamics during stand development after clear-cutting of Japanese cedar (*Cryptomeria japonica*) plantations

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Abstract:

We examined soil N dynamics, including inorganic N concentration, net N transformation rates, and estimated plant N uptake (EPNU) from soil N budgets, as well as litterfall inputs, in five Japanese cedar plantation stands of different ages (5, 16, 31, 42, and 89 years) in the Mt. Gomadan Experimental Forest (GEF). Net soil N mineralization and nitrification rates did not differ significantly between the youngest and oldest stands, while soil moisture and inorganic N concentration were higher in the youngest stand. The EPNU was highest in the 16-year-old stand and lowest in the 31-year-old stand, and had a significant negative correlation with litter C:N ratio. The oldest (89-year-old) stand had a higher soil C:N ratio, lower proportion of nitrification rate to mineralization rate (%NIT), and higher estimated plant NH_4^+ uptake than did the other stands, indicating that changes of soil organic matter quality can alter soil N dynamics. These results suggest that as a Japanese cedar plantation develops, soil N dynamics can be altered by the quantity and quality of input litter and soil organic matter, and can generate the imbalance between N supply from soil and N demand by plant..

41 **Introduction**

42

43 Internal nitrogen (N) cycling between plants and soil plays a very important role in N retention
44 of forested ecosystems (Vitousek and Howarth 1991; Davidson et al. 1992). One important
45 indicator of internal N cycling is soil N availability, which often limits net primary production
46 (NPP) in temperate forest ecosystems (Reich et al. 1997; Tateno et al. 2004). According to
47 previous research, soil N availability is the potential of a soil to produce the inorganic N (mainly
48 ammonium and nitrate N) available to plants. Soil N availability is regulated by the N
49 mineralization and nitrification rates, denitrification potential, and physical
50 absorption-desorption capacity in soil, as well as atmospheric N deposition (Pastor et al. 1984;
51 Davidson et al. 1992; Schimel and Bennet 2004; Booth et al. 2005). These factors are sensitive
52 to tree species composition (Lovett et al. 2004), litterfall quality (Hobbie 1992), soil moisture
53 and temperature (Stark and Firestone 1995), the quality of organic matter such as carbon (C) to
54 N (C:N) ratio (Lovett et al. 2004; Gundersen et al. 2006), climatic conditions (Dyer et al. 1990),
55 and soil composition (Hall et al. 2004; Page and Mitchell 2008).

56 Internal N cycling is also sensitive to natural and anthropogenic disturbances (Aber et al.
57 2002; Galloway et al. 2004; LeBauer and Treseder 2008). Increased concern about
58 human-related N loss in forest ecosystems has prompted research about how forest disturbance
59 affects internal N cycling and N retention systems (Vitousek et al. 1997; Gundersen et al. 2006).
60 Among the most serious disturbances is clear-cut harvesting, which causes large N losses via

61 biomass removal and hydrological N leaching (e.g., Bormann and Likens 1979; Aber et al.
62 2002), most likely due to the elimination of plant N uptake, and enhanced N mineralization and
63 nitrification rates in soils resulting from increased soil moisture and temperature (Likens et al.
64 1969; Vitousek et al. 1982; Fisk and Fahey 1990; Burns and Murdoch 2005). Although the
65 effects of forest disturbance on N cycling have been well-documented, the disturbance recovery
66 processes during subsequent re-establishment of forest stands have not yet been clarified
67 because long-term observations are required. Detailed knowledge of forest ecosystem recovery
68 processes is crucial to clarify the biogeochemical functions in forest ecosystems and to develop
69 better forest management strategies.

70 Long-term observations before and after a disturbance, as well as chronosequencial
71 approaches, are needed to evaluate how forests develop after the disturbance. Recovery
72 processes in forest ecosystems can be elucidated by comparing stands with similar features
73 except ‘stand age’ (i.e., years after disturbance and subsequent secondary growth or replanting;
74 Johnson and Miyanishi 2008). Several studies examining N cycling in a wide range of forest
75 ecosystems have used the chronosequence approach to document changes during forest
76 re-establishment after clear-cutting, wildfire, or land use change (e.g., Idol et al. 2003; White
77 et al. 2004; Bond-Lamberty et al. 2006; Yermakov and Rothtein 2006, Davidson et al. 2007).
78 However, these studies were unable to provide a sufficient explanation of how tree growth
79 processes affect N cycling, because they compared forest stands that differed in tree species
80 composition, soil properties, geology, or climatic conditions.

Artificial forests account for 41% of all forest area in Japan, and Japanese cedar *Cryptomeria japonica* D. Don is the dominant plantation species (comprising approximately 45% of the artificial forest area in Japan). These plantation forests have undergone, and will continue to undergo, intensive disturbances, such as clear-cutting. Thus, clarifying the biogeochemical functions of forest ecosystems in Japan requires determining how clear-cutting and subsequent stand development affects internal N cycling in Japanese cedar plantations. Tateno et al. (2009) described chronological changes in biomass accumulation patterns and internal N cycling by examining biomass allocation and plant nutrient status in Japanese cedar stands of various ages in the Mt. Gomadan Experimental Forest (GEF), central Japan. They found that soil N supply may limit plant growth and that N limitation may be enhanced under conditions of an imbalance between soil N supply and plant N demand 30 years after afforestation (Tateno et al. 2009). However, they did not explain the factors determining the changes in soil N dynamics.

In this study, we focused on soil N dynamics as an indicator of internal N cycling and the factors affecting these dynamics. We used the chronosequence approach to investigate changes in soil N dynamics during a development of Japanese cedar plantations. We compared soil N availability including inorganic N concentrations, mineralization and nitrification rates, and annual N budgets within soil, and litterfall input as factors affecting soil N dynamics among Japanese cedar stands of different ages. Our objectives were to clarify the patterns of soil N dynamics during stand development, and to elucidate possible mechanisms altering soil N

dynamics.

Materials and methods

Study site description

This study was conducted at the Mt. Gomadan Experimental Forest (GEF) in Nara Prefecture, central Japan (34˚04'N, 135˚35'E, Fig. 1). The watersheds within GEF consist primarily of plantations of Japanese cedar (*Cryptomeria japonica*), and small areas of Japanese cypress (*Chamaecyparis obtusa*) plantations along the ridge. Because forest management is conducted on a watershed basis, stand ages are identical within each watershed but differ among watersheds. In GEF, two-year-old conifer seedlings are planted the year after clear-cutting. Hereafter, we define the stand age in 2004 as the years since clear-cutting and subsequent replanting. After forest clear-cutting, slash and logging debris are gathered and arranged into strips, and seedlings are planted. Further details of forest management in GEF can be found in Fukushima and Tokuchi (2008; 2009).

The area overlays Cretaceous sedimentary rock composed of alternating sandstone, mudstone, and shale. Soils are classified as Cambisols or brown forest soils (Forest Soil Division 1976). Soil depth is shallow, in part because the forest slopes are very steep (average slope 30–70%; Fukushima and Tokuchi 2009). The mean annual temperature is 12.3°C, and the

121 mean annual precipitation is 2,521 mm (averaged 1976–2005), as measured at the Wakayama
122 Experimental Research Station, Field Science Education and Research Center (FSERC), Kyoto
123 University (34˚04'N, 135˚41'E, 533 m above sea level), located 4 km west of GEF (FSERC
124 2007). Snowpack varies interannually and spatially, but is usually continuous from January to
125 March, with depths < 0.7 m on the upper slopes.

126 Plant censuses were conducted in five watersheds: JC5, JC16, JC31, JC42, and JC89,
127 composed of 5-, 16-, 31-, 42-, and 89-year-old stands, respectively (Fig. 1). We established two
128 subplots (20 × 20 m) on lower slopes with a southerly aspect and surveyed stand characteristics
129 in 2002. Table 1 summarizes stand characteristics at each site.

130

131 Soil sampling

132 Soil samples were collected in the same watersheds as the plant censuses; sampling was
133 conducted in April, July, September, and December 2003 and April 2004 (Fig. 1).We dug soil
134 pits 50 cm deep and >1 m wide, with the exception of JC89, where soil did not extend below 30
135 cm because this layer contained mostly large gravel and cobbles and little fine soil. All soil pits
136 were dug within plant census plots. Three replicates of each soil layer (0–10, 10–30, and 30–50
137 cm mineral soil) were collected in each watershed using a stainless steel hand trowel.

138 In the field, the soil samples were divided in half. One half was placed in a clean
139 polyethylene bag in a cool box with refrigerant and brought to the laboratory at Kyoto
140 University. The other half was also placed in a clean polyethylene bag, but it was returned to its

141 original position for *in situ* incubation ('buried bag method,' Eno 1960). The buried samples
142 were dug up at the next soil sampling and brought to the laboratory. The incubation periods
143 were designated season I (April–July 2003; 68–77 days); season II (July–September 2003;
144 92–96 days); season III (September–December 2003; 65–70 days); and season IV (December
145 2003–April 2004; 140–142 days). All soil samples were sieved through a 2-mm mesh to break
146 up soil aggregates; gravels and fine roots were removed by hand and with tweezers. The treated
147 soil samples were stored at 4°C before analysis.

148 In addition to soil sampling, we used an ion exchange resin (IER) bag to determine the input
149 and output of ionized soil solutes in each soil layer of each soil pit (Giblin et al. 1994). Each
150 IER bag contained 7.5 g of anionic resins and 7.5 g of cationic resins within a polyvinyl chloride
151 (PVC) pipe (inner diameter 5 cm, depth 1 cm) and was tightly wrapped with water permeable
152 nylon mesh. Four IER bags were installed at depths of 10, 30, and 50 cm in each stand (the 50
153 cm depth was unavailable at JC89) and retrieved at the next soil survey. The retrieved resins
154 were opened, air-dried, and sealed prior to chemical analyses.

155 The soil bulk density of the samples was determined for each soil layer (0–10, 10–30, 30–50
156 cm; the 30–50 cm depth was unavailable at JC89) in each watershed in spring 2004, using 400
157 cm^2 soil blocks.

158

159 Soil chemical analysis

160 Fresh soil samples (2–3 g) were oven-dried at 105°C for 48 h to determine water content. A 5-g

(fresh mass) subsample of each soil sample was placed in a clean polyethylene bottle containing 50 mL 2 M KCl and shaken mechanically for 1 h to extract inorganic N. Extracts were filtered through Advantec No. 6 filter paper (Advantec MFS, Inc., Tokyo, Japan). Filtrates were frozen until colorimetric analyses for ammonium (NH_4^+) and nitrate (NO_3^-), which contains oxidized nitrite (NO_2^-) concentrations, using a BRAN+LUEBEE AutoAnalyzer III (BLTEC, Osaka, Japan). Soils incubated at the study sites were also extracted and analyzed as described above. The *in situ* net N mineralization rate during incubation was calculated as the net change in NH_4^+ and NO_3^- concentrations between the initial and incubated samples. *In situ* net nitrification was calculated as the net change in NO_3^- concentration. The remaining soil was air-dried prior to analysis for total C and N content, using an NC analyzer (NC-900, Shimadzu, Kyoto, Japan). Soil NH_4^+ and NO_3^- concentrations were described as mgN kg-dry-soil^{-1}, and net N mineralization and nitrification rates were described as mgN kg-dry-soil^{-1} per incubation period (seasons I–IV).

To measure absorbed N on the IER, a 5-g (air-dried mass) subsample of each resin was placed in a clean polyethylene bottle containing 100 mL 2 M KCl and shaken for 1 h. Extracts were filtered and analyzed for NH_4^+ and NO_3^- concentrations as described above. Filtered extracts were adjusted to pH 7.0 with 1 N NaOH before chemical analysis, as the pH of most IER extracts was very low (< 2.0).

Estimation of plant N uptake from soil N budgets

To calculate plant uptake of available inorganic N in soils, we used the following equation based on soil N budgets (Nadelhoffer et al. 1985; Tokuchi et al. 2002):

Estimated plant N uptake (EPNU; *i*-*j*) = produced N (*i*-*j*) + input N (*i*) - output N (*j*)- Δ N pool (*i*-*j*), (1)

where *i* and *j* indicate soil depth (cm; $i < j$), produced N (*i*-*j*) was determined from *in situ* net N mineralization at the *i*-*j* soil layer, input N (*i*) and output N (*j*) were determined from N captured on IER bags at depth *i* and *j*, respectively, and Δ N pool (*i*-*j*) was the net increment in soil inorganic N content at the *i*-*j* soil layer.

Nitrogen input into the uppermost soil layer (0 cm) was estimated from atmospheric or throughfall NO_3^- and NH_4^+ deposition data for 2005 and 2006 (Fukushima 2009). All terms in equation (1) are expressed as kgN ha^{-1} per a certain period, using soil bulk density. The calculation was performed for each soil layer and each N form (NH_4^+ and NO_3^-- N) during each season. Then annual plant N uptake was determined by summing all seasons throughout all soil layers. We used N mineralization rate to evaluate soil properties, and used EPNU as an indicator of internal N cycling in each stand.

Litterfall measurement

To estimate the mass of annual litterfall, ten 0.25-m^2 traps with 1-mm nylon mesh were installed

at a height of 1.3 m in all soil survey watersheds except JC5, where tree heights were too low (< 1.3 m). Five litter traps were randomly placed within the plant census plots (20 × 20 m), with one established on each bank of the stream in each watershed. Fallen litter was collected monthly from April 2003 to April 2004. Samples were dried at 40°C for at least 2 weeks, sorted into needles, branches, other materials (cones and seeds), and litter from other species, and then weighed. The C and N contents of needles were measured using an NC analyzer. We used the following chemical data as described by Igahara and Nakagawa (2002) and Inagaki et al. (2004) for branches, other materials, and other species: C content, 53.4%, 53.2%, and 51.9%; N content, 0.3%, 1.0%, and 1.5%, respectively.

Statistical analysis

A comparison of watersheds with the same climatic and geological conditions, such as those in GEF, enables straightforward analysis of how stand age affects N dynamics, and helps clarify stand development processes after clear-cutting and subsequent afforestation (Johnson and Miyanishi 2008; Tokuchi and Fukushima 2009). Our soil sampling design did not include replication of stand age, so our protocol of soil samples and IERs within a watershed constitutes pseudoreplication (Hurlbert 1984). This limits the discussion of how stand development affects soil N dynamics. However, Japanese cedar plantation forests under the same climatic and geologic conditions have a relatively homogeneous soil environment, despite the influence of topography-mediated conditions (Hirobe et al. 1998; Tokuchi et al. 2000; Ichikawa et al. 2003).

Thus, to compare soil N dynamics among forest watersheds of different ages, we established soil pits in each watershed at a similar position on a lower slope (i.e., a similar distance from a stream; Fig. 1), which allowed us to assess the effects of stand development in relation to the plant-soil-stream continuum and compare these effects with findings from previous studies conducted in GEF (Fukushima 2009; Fukushima and Tokuchi 2009; Tateno et al. 2009; Tokuchi and Fukushima 2009) and other areas (Bohlen et al. 2001; Johnson et al. 2000).

The effects of stand age on soil moisture, inorganic N concentrations, net N mineralization rate, and net nitrification rate were analyzed using one-way analysis of variance (ANOVA), followed by Scheffe's F test. The relationship between stand age and litterfall was analyzed using one-way ANOVA and Tukey's post-hoc test. We also used best-fit regression and correlation analysis to examine the relationship between the soil C:N ratio and percentage nitrification rate and between C and N input by litterfall and EPNU. All statistical analyses were conducted using SPSS 10.0J (SPSS 1999).

Results

Soil moisture, inorganic N concentrations, and net N mineralization and nitrification rates

239 Soil moisture at 0–10 and 10–30 cm depths was significantly higher in JC5 than in the other
240 stands at all soil depths ($P < 0.05$; Table 2). In deeper soil layers, soil moisture decreased in JC5
241 and increased in JC16 (Table 2). In JC31, JC42, and JC89, soil moisture did not differ
242 significantly among soil layers ($P > 0.05$; Table 2).

243 The *in situ* nitrification rate did not differ significantly among watersheds and soil layers, but
244 the *in situ* N mineralization rate differed significantly among soil layers in all watersheds,
245 except in JC89, the oldest stand, and among watersheds at 30–50 cm depths (Table 2). The
246 proportion of nitrified N to mineralized N (%NIT) exceeded 100% in almost all soil layers,
247 indicating that NH_4^+ present at the start of the *in situ* incubation was nitrified (Table 2). The
248 lowest %NIT was observed at 0–10 cm depth in JC89 (70%).

249

250 Soil N budgets and estimated plant N uptake

251 Table 3 lists annual EPNU, input N, output N, produced N, and changes in N content. The JC5
252 stand received a total N deposition of 9.3 kgN ha^{-1} yr^{-1}. The closed-canopy JC31, JC42, and
253 JC89 stands received total deposition of 13.9 kgN ha^{-1} yr^{-1} via throughfall. For the JC16 stand,
254 where the forest canopy was half-open, we used the arithmetic mean value between rainfall and
255 throughfall (11.6 kgN ha^{-1} yr^{-1} in total N). Output N from the bottom layer, estimated from
256 extractable IER-captured NH_4^+ and NO_3^-, was 23.5, 4.3, 12.0, 6.3, and 21.0 kgN ha^{-1} yr^{-1}, and
257 mineralized N (i.e., produced N) in all soil layers was 30.6, 46.2, 21.3, 18.7, and 42.0 kgN ha^{-1}
258 yr^{-1} in JC5, JC16, JC31, JC42, and JC89, respectively. Based on these results, the EPNU was

18.0, 53.4, 24.8, 24.2, and 34.2 kgN ha^{-1} yr^{-1} in JC5, JC16, JC31, JC42, and JC89, respectively (Table 3). Estimated plant uptake of NO_3^- was highest in JC16, and that of NH_4^+ was highest in JC89.

Soil C:N ratio

The soil C:N ratio ranged from 10.8 to 16.6 and decreased at each stand as soil depth increased (Table 4; Tateno et al. 2009). The highest values were observed in JC89 (16.6 and 16.0 at 0–10 and 10–30 cm depths, respectively). Soil C:N ratio (CN_{soil}) and %NIT (Fig. 2) exhibited a significant non-linear relationship, fitted by an exponential curve ($R^2 = 0.580$, $P < 0.001$) as follows:

$$[\%NIT] = 1.34 \times 10^6 \times [CN_{soil}]^{-3.58} \quad (2).$$

Litterfall input

Annual litterfall mass was lowest in JC16 (2.5 t ha^{-1} yr^{-1}), highest in JC31 and JC42 (4.9 t ha^{-1} yr^{-1}), and intermediate in JC89 (4.2 t ha^{-1} yr^{-1}; Fig. 3). Of the litter composition from all stands, needle litterfall was significantly greater in the three older stands (JC31, JC42, and JC89) than in the young stand (JC16). Branch litterfall was approximately three times greater in middle-aged stands (JC31 and JC42) than in the young stand (JC16). The amount of other materials, including cones and seeds, decreased in the following order: JC89 > JC42 > JC31 = JC16. Litter from other species, including broad-leaved deciduous trees and annual herbaceous

279 plants, was significantly higher in JC16 than in the other stands.

280 Table 5 summarizes annual litterfall C and N amounts and the annual mean C:N ratio of
281 litterfall. Both total C and N inputs and C:N ratio were higher in the middle-aged stands (JC31
282 and JC42) than in the younger (JC5) and older (JC89) stands. The relationship between EPNU
283 and annual C input via litterfall was significantly negative ($R^2 = 0.996$, $P < 0.001$; Fig. 4a), with
284 its r-squared being greater than that between annual litter N input and EPNU ($R^2 = 0.919$, $P <$
285 0.001). The correlation between EPNU and C:N ratio of litterfall was also significantly negative
286 ($R^2 = 0.958$, $P < 0.001$; Fig. 4b).

287

288

289 **Discussion**

290 Effects of forest clear-cutting on soil N dynamics

291 Many studies have reported that when forests are clear-cut, decreased plant N uptake and
292 enhanced microbial activity, such as that by autotrophic nitrifiers, result in high levels of NO_3^-
293 leaching and loss in soil water and streamwater (Likens et al. 1969; Vitousek et al. 1982; Fisk
294 and Fahey 1990; Bradley 2001; Burns and Murdoch 2005). In GEF, stream NO_3^- concentrations
295 increased and peaked about three years after clear-cutting (Fukushima and Tokuchi 2008).
296 However, we observed no significant increases in net N mineralization and nitrification rates in
297 soil, despite the significantly higher soil moisture, especially at the 0–10 cm depth (Table 2),
298 which is an important factor affecting soil microbial activity (Stark and Firestone 1995). Fisk

and Fahey (1990) reported that the enhanced net nitrification potential due to clear-cutting of northern hardwood forests in eastern North America began to decline two years after clear-cutting. Burns and Murdoch (2005) reported no increases in N mineralization rate or nitrification rate in clear-cut northern hardwood forests, as soil nitrification rates were high before clear-cutting. Also, the absence of increased soil net N mineralization or nitrification rates in JC5 at GEF may indicate that clear-cutting has a minimal effect on these rates, or that these effects last no longer than five years after clear-cutting. This JC5 watershed had significantly higher soil moisture and soil NO_3^- content than other stands at the surface (0–10 cm depth) in almost all seasons, including summer, when the nitrification rate in all stands was high (Appendix A and B in the electronic supplementary material). Diminished plant activity (i.e., nutrient uptake and evapotranspiration rate) in JC5 could explain this, suggesting that the cessation of plant N uptake may be the primary cause for the increased stream NO_3^- concentration after clear-cutting (Fukushima and Tokuchi 2008).

Changes in soil N dynamics during forest development

Previous studies conducted in GEF have reported little change in atmospheric N input and stream NO_3^- concentration in stands more than 16 years old (Fukushima and Tokuchi 2008; Fukushima 2009). In contrast, biomass accumulation rates increase after clear-cutting, peaking in stands about 30 years old and subsequently declining (Tateno et al. 2009).

With regard to soil N dynamics, EPNU exhibited non-linear trends as stand age increased in

319 stands older than 16 years (Table 3). The JC16 stand exhibited the highest net N mineralization
320 rate in all soil layers, resulting in the largest EPNU (Table 3, Appendix A in the electronic
321 supplementary material). A possible factor determining this high mineralization rate in JC16 is
322 the litter quality. A greater contribution from leaf litter of other species, including broad-leaved
323 deciduous trees and annual herbaceous plants, which likely have higher N concentrations than
324 Japanese cedar needles, caused the lower C:N ratio of annual litterfall in JC16 (Table 5; Fig. 3).
325 Many studies have reported that input litter with a high N concentration and a low C:N ratio
326 could rapidly release inorganic N during the decomposition process and promote N
327 mineralization in N-limited forest soil (Hobbie 1992; Chapin et al. 2002). This finding suggests
328 that input of litter with a high N concentration could elevate the soil N status in JC16, and that
329 the high supply of available inorganic N in the soil may support the early growth of planted
330 Japanese cedars.

331 In contrast, plant N uptake estimated from the soil N budget declined remarkably in JC31
332 (Table 3), where Japanese cedars had the highest growth rate (i.e., productivity) of all the stands
333 (Tateno et al. 2009). As reported by Tateno et al. (2009), an imbalance in the available N supply
334 from soil (plant N uptake estimated from soil N budget) and plant N demand (biomass growth
335 rate) can accelerate the N limitation status. This phase is crucial to elucidate the changes in
336 internal N cycling during the development of Japanese cedar plantations.

337 An alteration of soil N dynamics in JC31 could be driven by both litter quantity and quality
338 (Table 5). We found the negative relationship between EPNU and litterfall C (Fig. 4a) and N

input. In contrast, previous studies reported that the positive relationship (Pastor et al. 1984; Nadelhoffer et al. 1985; Tateno et al. 2004) or little relationship (Scott and Binkley 1997; Enoki et al. 1997) between EPNU or soil N mineralization and litterfall. They discussed that higher productive forest which produces larger amounts of litterfall would tend to be established on the soil of higher N availability. In GEF, however, we also found a significantly negative correlation between EPNU and litter C:N ratio (Fig. 4b). During decomposition of organic materials with a higher C:N ratio, heterotrophic soil microbes demand more N, resulting in a reduced net nitrification rate and/or a reduced proportion of nitrification to mineralization (Aber et al. 2003; Lovett et al. 2004). Moreover, at the beginning of the fresh litter decomposition process, labile C (such as water-soluble polysaccharides) leaches, as demonstrated by Nioh and Kutuna (1989), and it can accelerate microbial N immobilization (Chapin et al. 2002). In addition, slowly decomposing branches comprise a significantly larger fraction of total litterfall in middle-aged stands (Fig. 3), which may enhance N immobilization rates during their decomposition, as reported by Vitousek et al. (1988). Therefore, although the relative importance of quality versus quantity of litterfall to affect soil N availability is still unknown, these results indicate that an increase in amounts of litterfall with higher C:N ratio and with slowly decomposing fraction suppressed soil nitrification activity, and EPNU decreased in JC31.

Prescott (2002) reported that the canopy plays a key role in soil N processes as a litter producer. Because of the physical traits of Japanese cedar, the difference between its tree height and crown length (‘clear-length’) increases with increasing tree height after canopy closure

(Kanazawa et al. 1985; Takeshita 1985; Chiba et al. 1990). As a result, the fall of relatively large branches with dead needles increases. In GEF, canopy closure typically takes about 20–30 years, during which branch litterfall increases (Fig. 3). This quantitative and qualitative change of litterfall likely altered soil N dynamics and EPNU in a non-linear manner, and as a result biomass productivity declined.

The %NIT was high overall in GEF (more than 100%; Table 2); this is consistent with the findings of Hirai et al. (2006), who compiled data on soil N transformation rates in Japanese cedar forests across Japan. Of them, lower %NIT values and a higher contribution of estimated plant NH_4^+ uptake to total N uptake indicated that NH_4^+ dominated soil N dynamics in JC89. Moreover, of the inorganic N forms that serve as plant nutrients, NH_4^+ made a relatively higher contribution to soil inorganic N concentration in JC89 (Table 2). This is partly because the C:N ratio of soil organic matter was higher in JC89 than in the other stands (Table 4). In GEF, the %NIT was significantly and inversely correlated with mineral soil C:N ratios (Fig. 2). Similar trends between soil C:N ratios and net nitrification rate or %NIT have been reported across eastern North American (Goodale and Aber 2001; Aber et al. 2003) and European (MacDonald et al. 2002) forests, as well in Japanese cedar forests (Hirobe et al. 1998); this relationship can be explained as described above. Thus, an increase in soil C:N ratio with stand age after forest canopy closure is likely to result in NH_4^+ dominating in soil N dynamics.

White et al. (2004) reported that the net N mineralization in mineral soil following clear-cutting and fire disturbances declined for the first 20 years in bigtooth aspen (*Populus*

379 *grandidentata*) forests, and then increased toward a maximum in the oldest (87-year-old) stand.
380 These patterns were positively correlated with biomass accumulation, indicating that soil N
381 dynamics can support continuous tree growth as stand age increases (White et al. 2004). In
382 contrast, Tateno et al. (2009) reported that even a re-increase of net N mineralization in JC89 of
383 GEF did not elevate the growth rate. One possible explanation for these conflicting findings
384 may be related to the form of N preferred by plants (Haynes and Goh 1978; Nordin et al. 2001).
385 Koba et al. (2003) investigated natural $\delta^{15}N$ in plants and soils in a Japanese cedar plantation,
386 and reported that Japanese cedars would prefer NO_3^-, particularly in high-nitrification sites on
387 lower slopes of Mt. Ryuoh, central Japan, where soil properties are similar to those in GEF. In
388 the present study, changes in the form of soil inorganic N in an old Japanese cedar stand (with
389 elevated NH_4^+ availability) were shown, which may likely cause plant growth to remain limited
390 by N.

391 Another possibility is the contribution of soil microbes to N dynamics. As soil C:N
392 increases, N immobilization by soil microbes becomes an important pathway in soil N processes
393 (Davidson et al. 1992; Kaye and Hart 1997; Bengtsson et al. 2003). In older GEF stand with
394 higher soil C:N ratio, competition for inorganic N between plants and soil microbes may be
395 more intense than in younger stands. As a result, Japanese cedars likely remain N limited even if
396 EPNU increased in the old stand. These possibilities, however, cannot directly explain the
397 reasons for the conflicts between the EPNU in soil and the growth rate of cedar in JC89. Further
398 research is needed to elucidate the preference of Japanese cedar for NH_4^+ or NO_3^- as an N

source and more detailed soil N dynamics presented by gross N transformation rate.

In conclusion, our findings reveal that as a Japanese cedar plantation develops, soil N dynamics are affected by both the quantity and the quality of input litter. A decline in plant N uptake, estimated from the soil N budget, is likely driven by plant canopy closure. These changes in soil N dynamics can generate the imbalance between N supply from soil and N demand by N.

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579

580 **Figure legends**

581

582 Figure 1. Study site location and target watersheds of this study. The stand age of each of the
583 five watersheds is shown, with the watershed number given in parentheses. Black squares
584 indicate the locations of plant census plots. Soil sampling was conducted within the left-bank
585 plot.

586

587 Figure 2. Relationship between soil C:N and percent nitrification (%NIT).
588 %NIT was calculated as net nitrification rate divided by net N mineralization rate, indicated as a
589 percentage.

590

591 Figure 3. Annual amount and composition of litterfall. Different letters indicate significant
592 differences among watersheds of each litter component ($P < 0.05$, ANOVA, Tukey's post hoc
593 test; a, b: needle litter; A, B: branch litter; x, y, z: others' litter; X, Y: other leaf litter).

594

595 Figure 4. Relationship between estimated plant N uptake (EPNU) and C input via litterfall (a),
596 and C:N ratio of litterfall (b). EPNU = -0.023*[C input] + 83.5 ($R^2 = 0.9958$, $P < 0.001$), and
597 EPNU = -0.988*[C:N ratio] + 103.5 ($R^2 = 0.9958$, $P < 0.001$).

598

599 **Tables**

Table 1. Stand characteristics and structure of different aged Japanese cedar of five watersheds.

Watershed	JC5	JC16	JC31	JC42	JC89
Tree density (n ha^{-1}) *a*	3450	3250	3013	2300	1725
Mean d.b.h. (cm) *a*	2.2	6.9	14.2	18.5	23.1
Above- and below-ground biomass (t ha^{-1}) *b*	0.9	41.2	183.1	293.4	346.7
Canopy status *c*	Full-open	Half-open	Closed	Closed	Closed

a; data from Fukushima and Tokuchi (2008), *b*; data from Tateno et al. (2009), *c*; data from personal communications (K. Fukushima).
d.b.h. indicates the diameter at breast height.

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Table 2. Mean soil moisture (g g^{-1}), NH_4^+ and NO_3^- concentrations (mgN kg^{-1}), *in situ* net mineralization and nitrification rates (mgN kg^{-1} $period^{-1}$), and percent nitrification (fraction of mineralized N converted to nitrate; %) in each soil layer of the five watersheds over four observations. Standard errors are given in parentheses.

Watershed	JC5		JC16		JC31		JC42		JC89	
Soil moisture (g $g\text{-}soil^{-1}$)										
0-10 cm	0.678	(0.004) A	0.516	(0.010) B	0.539	(0.013) B	0.506	(0.006) B	0.546	(0.011) B
10-30 cm	0.638	(0.002) A	0.547	(0.010) BC	0.503	(0.016) C	0.502	(0.006) C	0.570	(0.012) B
30-50 cm	0.560	(0.005) A	0.554	(0.009) A	0.476	(0.025) B	0.495	(0.006) B		
NH_4^+ content (mgN $kg\text{-}dry\text{-}soil^{-1}$)										
0-10 cm	15.26	(1.58) A	7.71	(0.85) B	10.75	(1.64) AB	8.70	(1.79) AB	11.86	(1.65) AB
10-30 cm	13.70	(1.90)	10.11	(1.64)	11.76	(2.03)	11.30	(2.14)	12.22	(0.42)
30-50 cm	9.69	(0.81)	11.38	(1.42)	10.91	(1.83)	9.72	(1.67)		
NO_3^- content (mgN $kg\text{-}dry\text{-}soil^{-1}$)										
0-10 cm	5.82	(0.80) A	1.25	(0.30) B	3.90	(0.50) A	1.77	(0.21) B	0.43	(0.03) B
10-30 cm	2.99	(1.46)	1.62	(0.36)	1.55	(0.31)	1.29	(0.18)	2.15	(0.55)
30-50 cm	2.33	(1.03)	2.09	(0.29)	1.22	(0.30)	1.57	(0.56)		
Net mineralization rate (mgN $kg\text{-}dry\text{-}soil^{-1}$ $period^{-1}$)										
0-10 cm	26.87	(7.19)	32.97	(7.57)	23.40	(5.07)	23.94	(7.14)	27.54	(12.80)
10-30 cm	6.35	(3.32)	8.67	(3.72)	2.60	(2.23)	0.83	(2.73)	13.55	(3.30)
30-50 cm	1.84	(1.24) B	10.21	(2.78) A	1.33	(0.98) B	1.44	(2.32) B		
Net nitrification rate (mgN $kg\text{-}dry\text{-}soil^{-1}$ $period^{-1}$)										
0-10 cm	28.04	(6.07)	27.48	(7.65)	24.11	(4.80)	21.39	(8.05)	19.26	(14.14)
10-30 cm	6.44	(1.66)	9.86	(2.79)	5.71	(1.21)	4.19	(0.66)	12.45	(2.92)
30-50 cm	2.81	(0.57)	9.62	(2.70)	4.30	(2.04)	2.60	(0.21)		
Percent nitrification (%)										
0-10 cm	104.3		83.3		103.0		89.3		70.0	
10-30 cm	101.5		113.7		219.6		503.0		91.9	
30-50 cm	152.7		94.2		323.4		181.1			

Different large letters indicate significant differences among watersheds (A, B, C; $P < 0.05$, one-way ANOVA, followed by Scheffe's *F* test). No letter indicates no significant difference. Statistical analysis on seasonality and stand age effects by two-way ANOVA was summarized in Appendix C in the electronic supplementary material.

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Table 3. Annual estimated plant N uptake (EPNU), input N, output N, production N, and change in soil N content.

Watershed		EPNU	Input *c*	Output *d*	Production *e*	⊿pool *f*
		(kgN ha^{-1} yr^{-1})				
JC5 *a*	NH_4^+	3.8	5.8	2.3	-1.8	-2.1
	NO_3^-	14.2	3.5	21.2	32.4	0.6
	TIN	18.0	9.3	23.5	30.6	-1.5
JC16 *a*	NH_4^+	6.1	5.5	2.3	2.1	-0.8
	NO_3^-	47.3	6.1	2.0	44.1	0.9
	TIN	53.4	11.6	4.3	46.2	0.1
JC31 *a*	NH_4^+	-4.0	5.2	2.6	-8.6	-1.9
	NO_3^-	28.8	8.7	9.5	29.9	0.3
	TIN	24.8	13.9	12.0	21.3	-1.7
JC42 *a*	NH_4^+	-3.5	5.2	2.3	-5.2	1.2
	NO_3^-	27.7	8.7	4.1	23.9	0.8
	TIN	24.2	13.9	6.3	18.7	2.0
JC89 *b*	NH_4^+	9.1	5.2	4.0	7.4	-0.5
	NO_3^-	25.2	8.7	16.9	34.6	1.2
	TIN	34.2	13.9	21.0	42.0	0.7

TIN; total inorganic nitrogen.
a; 0-50 cm depth, *b*; 0-30 cm depth, *c*; Input is annual N input via precipitation or thoughfall by Fukushima (2009). *d*; Output is annual N captured in IER bag in the bottom soil layer . *e*; Production is annual mineralized or nitrified N estimated by buried bag method. *f*; ⊿pool is annual change in soil N. See text and equation (1).

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Table 4. C:N ratio of soil organic matter collected on four sampling dates.

Watershed	JC5	JC16	JC31	JC42	JC89
Depth (cm)					
0-10	13.2	13.8	13.7	13.5	16.6
10-30	13.0	13.2	11.5	12.1	16.0
30-50	12.8	13.2	10.8	11.2	N.A.

N.A., not available.

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Table 5. Annual organic matter (OM), C, and N input via litterfall and weighted mean C:N ratio of litterfall in each watershed.

Watershed	JC16	JC31	JC42	JC89
OM (t ha^{-1} yr^{-1})	2.6	4.9	4.9	4.2
C (kgC ha^{-1} yr^{-1})	1326	2530	2621	2098
N (kgN ha^{-1} yr^{-1})	26.0	33.5	31.8	29.4
C : N ratio	51	75	82	71

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618 **Legends of Appendix in the electronic supplementary material**

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620 Appendix A. Means of (a) soil moisture, (b) NH_4^+ concentration, (c) NO_3^- concentration, (d) net
621 N mineralization rate, and (e) net nitrification rate in each watershed during all seasons. Bars
622 indicate standard errors (*n*=3).
623 Left column: 0-10 cm depth; middle column: 10-30 cm depth; right column: 30-50 cm depth.

624

625 Significant but complex differences appeared among both watersheds and seasons in soil
626 moisture, NH_4^+ and NO_3^- concentrations and net N mineralization and nitrification rates
627 (Statistical results in Appendix B). In JC5, the youngest stand, soil moisture was higher than in
628 other stands throughout all seasons, and NO_3^- concentration was remarkably high in the summer
629 (season II) in all layers. On the whole, at 0–10 and 10–30 cm depths, soil NH_4^+ and NO_3^-
630 concentrations tended to be higher in the summer (season II) and/or dormant (season IV)
631 periods than in other seasons. At the 0–10 cm depth, two-way ANOVA revealed relatively high
632 N mineralization and nitrification rates in summer (season II), regardless of stand age.

633

634

635 Appendix B. Results of two-way ANOVA of soil moisture, NH_4^+ and NO_3^- concentrations, net N
636 mineralization rate, and net nitrification rate between stand age and season.

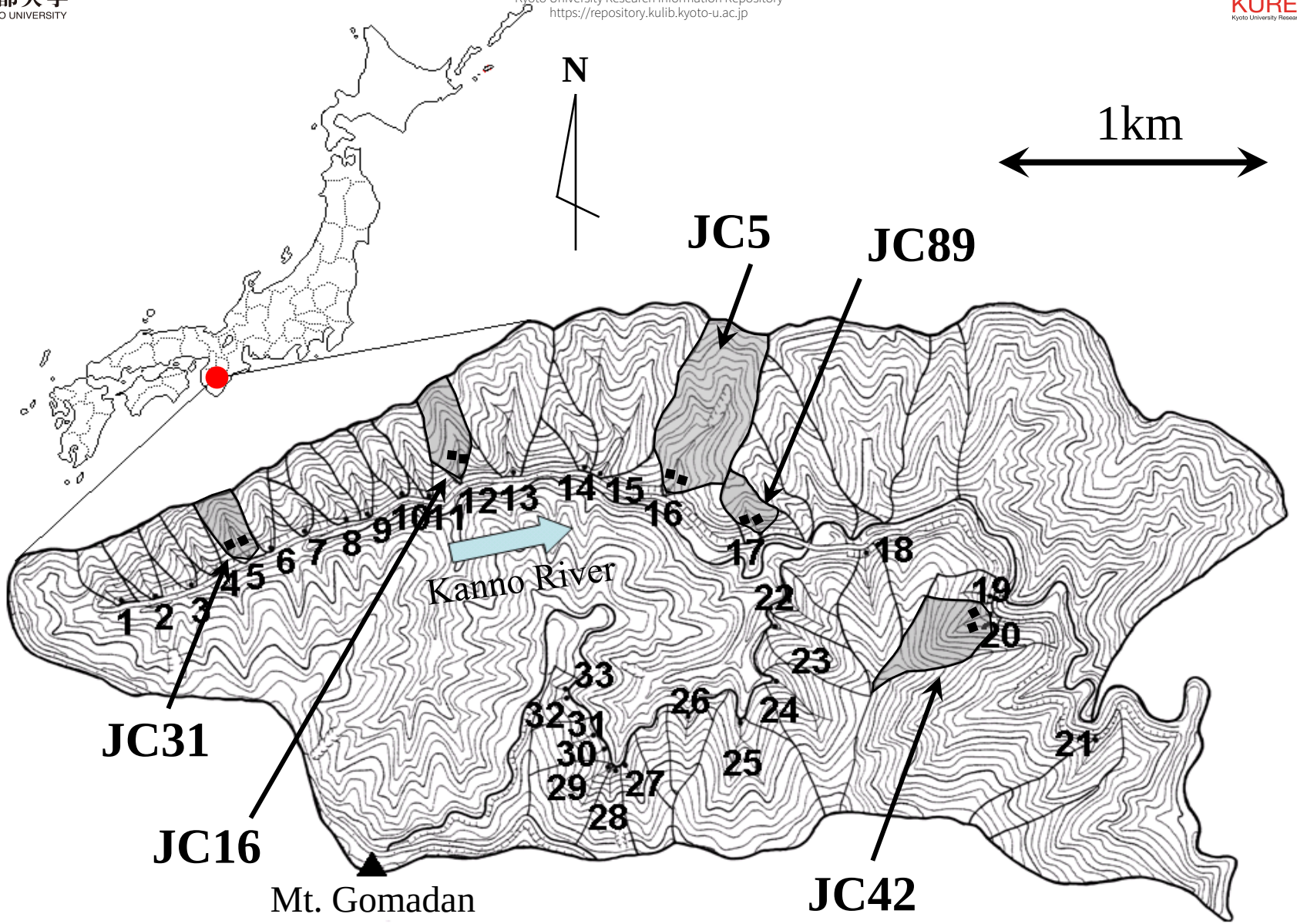


Fig. 1, Fukushima et al.

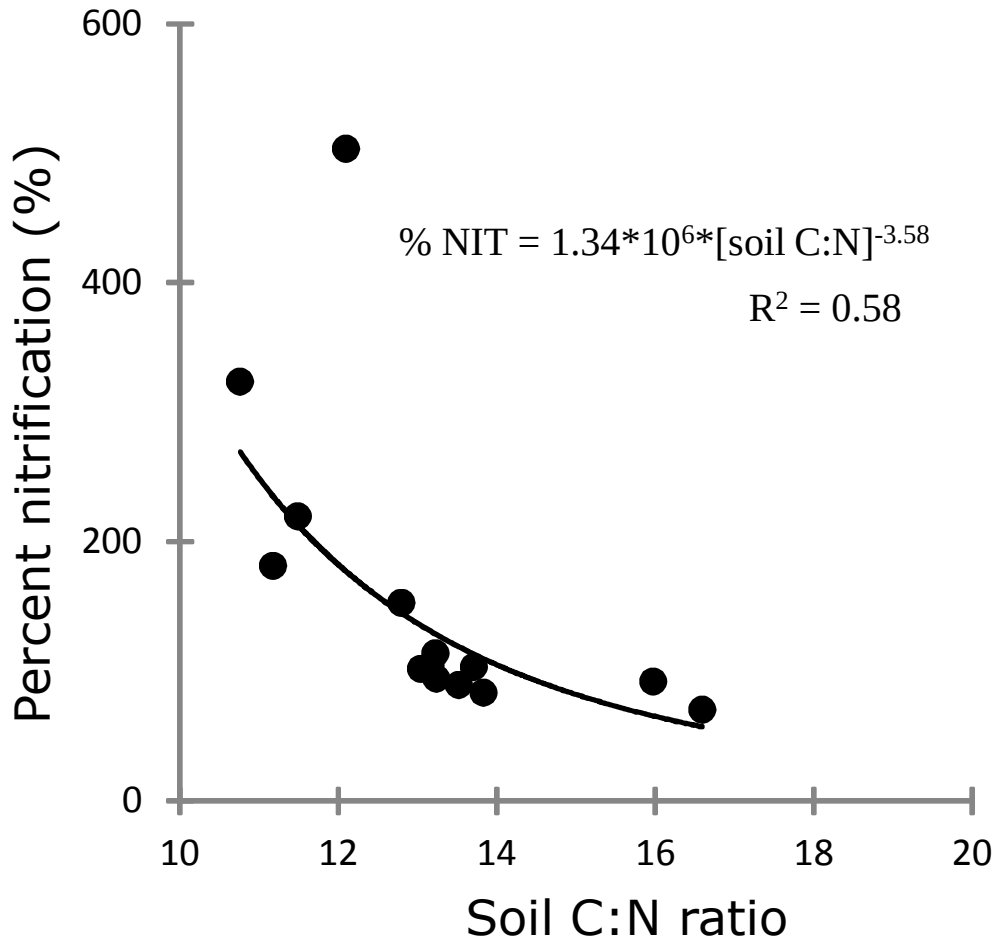


Fig. 2, Fukushima et al.

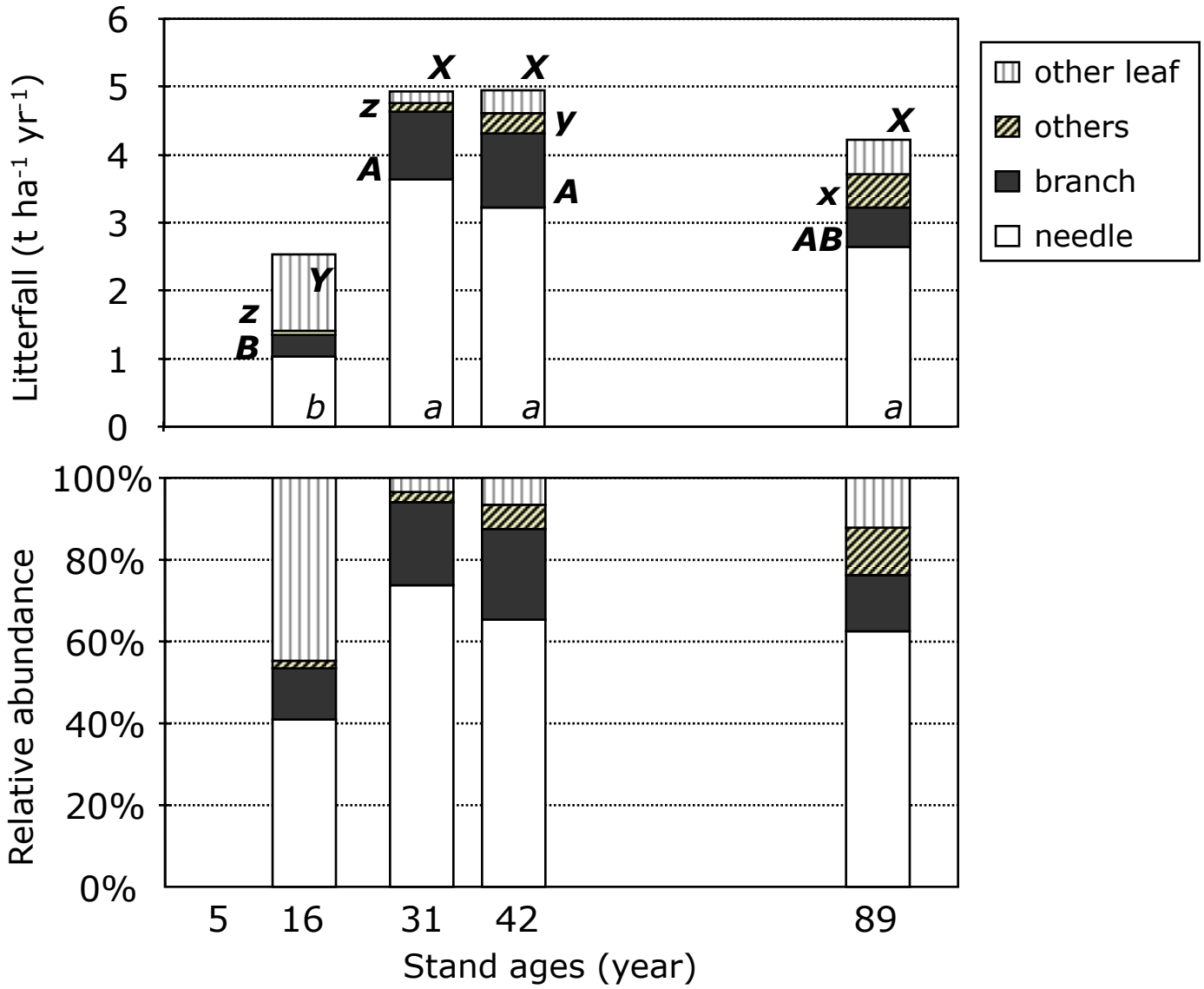


Fig. 3, Fukushima et al.

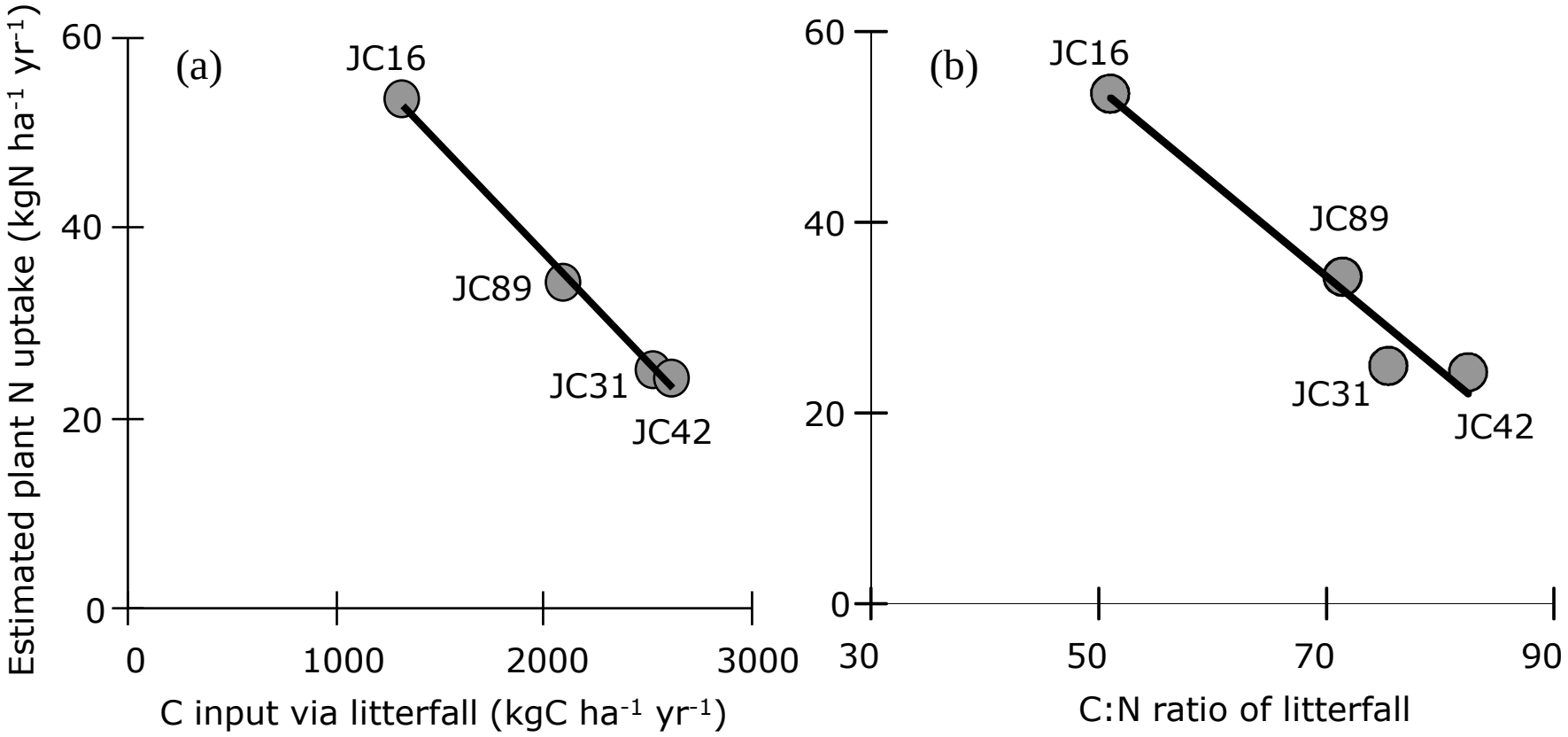
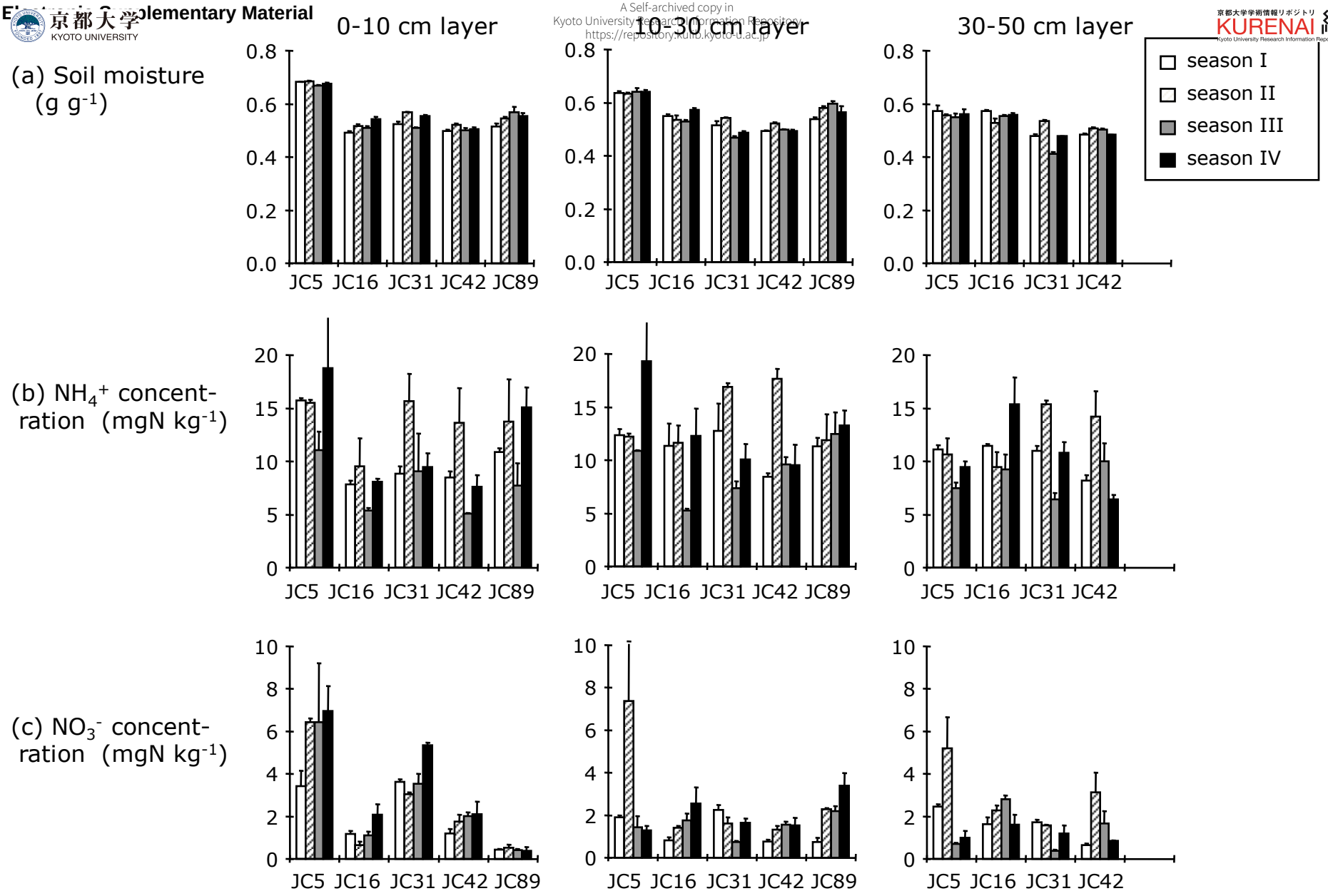
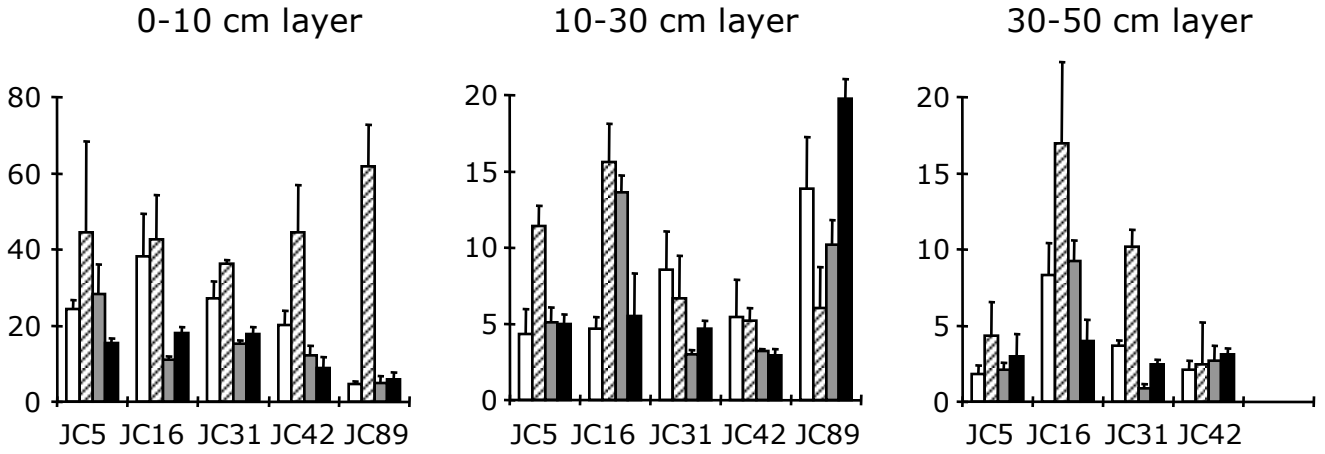


Fig. 4, Fukushima et al.

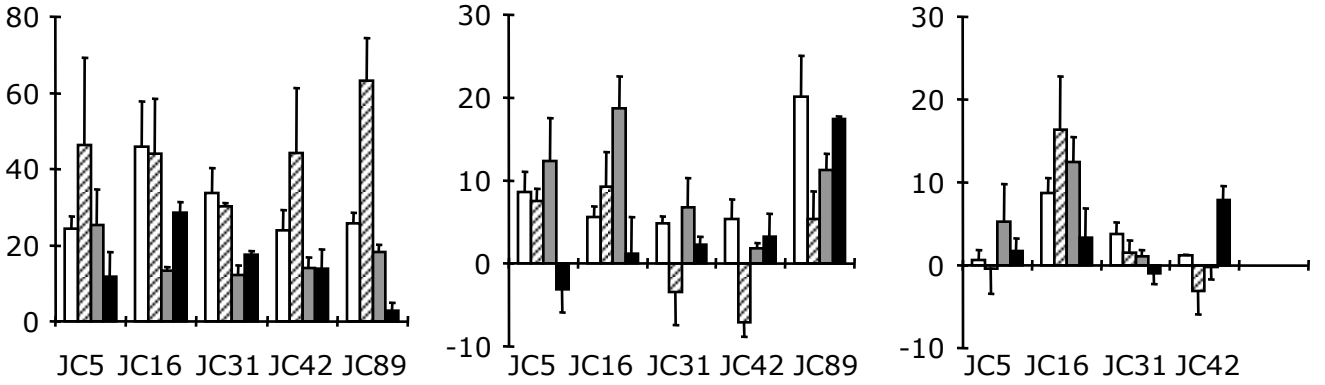


Appendix A., Fukushima et al.

(d) Net N mineralization rate ($mgN\ kg^{-1}\ period^{-1}$)



(e) Net nitrification rate ($mgN\ kg^{-1}\ period^{-1}$)



Appendix A (cont.), Fukushima et al.

Appendix B. Results of two-way ANOVA of soil moisture, NH_4^+ and NO_3^- concentrations, net N mineralization rate, and net nitrification rate between stand age and season.

	0-10 cm				10-30 cm				30-50 cm			
	df	*F*	*P*		df	*F*	*P*		df	*F*	*P*	
Soil moisture (g g-soil^{-1})												
age	4	296.62	<0.0001	***	4	139.18	<0.0001	***	3	73.973	<0.0001	***
season	3	11.286	<0.0001	***	3	3.0397	0.0399	*	3	6.0209	0.0023	**
season*age	12	4.0812	0.0004	***	12	5.3389	<0.0001	***	9	8.8317	<0.0001	***
NH_4^+ concentration (mgN kg-dry-soil^{-1})												
age	4	7.6746	0.0001	***	4	2.663	0.0467	*	3	1.9399	0.143	ns
season	3	6.9101	0.0007	***	3	7.8302	0.0003	***	3	7.6924	0.0005	***
season*age	12	0.8641	0.5879	ns	12	3.3046	0.0023	**	9	5.2357	0.0002	***
NO_3^- concentration (mgN kg-dry-soil^{-1})												
age	4	35.58	<0.0001	***	4	4.1231	0.007	**	3	4.0002	0.0159	*
season	3	3.1483	0.0354	*	3	4.2822	0.0105	*	3	11.462	<0.0001	***
season*age	12	1.119	0.3725	ns	12	4.2531	0.0003	***	9	4.4093	0.0008	***
Net N mineralization rate (mgN kg-dry-soil^{-1} period^{-1})												
age	4	0.7368	0.5724	ns	4	10.994	<0.0001	***	3	10.266	<0.0001	***
season	3	12.909	<0.0001	***	3	7.6351	0.0004	***	3	0.2604	0.8534	ns
season*age	12	1.1926	0.3213	ns	12	3.3009	0.0022	**	9	2.7452	0.0169	*
Net nitrification rate (mgN kg-dry-soil^{-1} period^{-1})												
age	4	0.9235	0.4599	ns	4	13.6	<0.0001	***	3	12.679	<0.0001	***
season	3	18.455	<0.0001	***	3	1.1483	0.3414	ns	3	7.1234	0.0008	***
season*age	12	1.5199	0.1574	ns	12	6.0435	<0.0001	***	9	2.2753	0.0422	*

*; $P < 0.05$, **; $P < 0.01$, ***; $P < 0.001$, ns; not significance ($P > 0.05$).